

Original Research Article

Heavy Metal Removal from Electronic Waste Polluted Water Using Indigenous Fungi and Its Implications on Public Health

Comment [GA1]: Are you trying to communicate the public health implications of the indigenous Fungi post removing heavy metals or the public health implication of being able to remove heavy metals by indigenous fungi.

ABSTRACT

Background: Heavy metals (HM) pollutants are crucial environmental and public health problems due to their toxicity, which has implications on public health.

Aim: This study was carried out to determine the biosorption efficiency of indigenous fungi isolates in reducing heavy metal present in electronic wastes polluted water body.

Study Design: This is a laboratory based study

Place and Duration of Study: The study was conducted at the Microbial Resources Research Laboratory, Department of Pure and Applied Biology, Ladoké Akintola University of Technology Ogbomoso, Nigeria from July 2021 to June 2022.

Methodology: Heavy metal polluted water sample was collected near the dumpsite of e-waste in Alaba International market, Lagos Nigeria. Fungi were isolated from the polluted water sample by carrying out serial dilution. Pure colonies were obtained and stored at 4 °C. Media formulations (MF) trials for the biosorption process was achieved using brewery waste and honeycomb extracts. Exactly 200 ml of MF were dispensed into a 500 ml Erlenmeyer flask containing 100 ml of the e-waste polluted water and fungal discs. Atomic absorption spectrophotometer was used to determine the heavy metal concentration in the water samples. The biological interactions of the fungi with the polluted water sample was monitored using Fourier transform infrared (FTIR) spectroscopy. The pH, electrical conductivity and other physicochemical parameters were also determined.

Results: *Trichoderma harzianum*, *T. viride*, *Fusarium oxysporum*, *Aspergillus flavus* and *A. niger* were isolated from the heavy metal polluted water samples. Heavy metal such as Pb (13.30 mg/L), Cd (16.50 mg/L), Cr (6.41 mg/L), Ni (3.81 mg/L), Zn (8.85 mg/L), Cu (8.33 mg/L), Fe (5.60 mg/L) had values which were higher than the acceptable limits. Biosorption efficiency of each of the fungus in reducing the metals present in the sample was in the increasing order of *A. niger* < *A. flavus* < *F. oxysporum* < *T. viride* < *T. harzianum*. FTIR showed that some peaks were shifted to lower wavenumber as a result of the interactions of the fungus with the heavy metal in the water sample. This study revealed that *T. harzianum* had the highest biosorption efficiency for the removal of heavy metal in the polluted water sample.

Conclusion: It is essential that heavy metals be removed from polluted water body to avoid its deleterious effects on public health. This can be achieved biologically using any of the fungi isolated in this work.

Comment [GA2]: Include in the background why you think the study location has heavy metal contamination – has there been report in the news? Previous studies done? Public health reports of contamination? Complete a Conceptual Site Model showing the source, pathway and receptor linkages before your intervention.

Comment [GA3]: Abilities first before efficiency

Comment [GA4]: Was

Comment [GA5]: How did you determine that the water already had heavy metal pollution or this was just an assumption?

Comment [GA6]: Read again, too long.

Comment [GA7]: Why was this done? For the benefit of the microorganisms?

Comment [GA8]: So the approach is normally to assay the water for heavy metal contamination and then report the concentration of heavy metals periodically till the end of the study. The preliminary analyses should be presented first to confirm there is indeed heavy metal contamination.

Keywords: E-waste polluted water, Heavy metal, Fungi, FTIR

1. INTRODUCTION

Environmental pollution arises due to industrialization and mining of natural resources leading to the contamination of soil, air, sediments and surface water with heavy metals [1]. Apart from these, human activities also lead to situations in which heavy metals are transformed into new compounds and may be spread worldwide [2]. Hence, there is a need for new techniques that focuses on the removal of the pollutants instead of the conventional methods of disposal that results in environmental pollution [3,4] Heavy metals can be divided into three based on their physiological effect and toxicity as low toxicity (Fe, Mo, Mn), average toxicity (Zn, Ni, Cu, V, Co, W, Cr) and high toxicity (As, Ag, Sb, Cd, Hg, Pb, U) [5].

Metal collections such as Lead, Cadmium, Chromium, Copper, Cobalt, Mercury and Nickel as well as Zinc generate dangerous effects on human health when amounts that cannot be processed are absorbed when discharged through human activities such as mining, waste disposal or industrial discharge. However, heavy metals, such as cadmium were not known to be dangerous until the early 19th century [2]. When found in the body system, they result in severe health problems which hamper normal functions [6]. Chronic exposure occurs which refers to contact with low levels of heavy metal over a long period [2]. The toxicity effects greatly depend on the proportion of the contaminant present in the environment in the form(s) that can be assimilated by the organism [7]. Exposure for a long duration and heavy metals accumulation often present dangerous health consequences on aquatic and human life [8, 9].

There is a short life span of electronic products as a result of higher demands for newer and more efficient technologies leading to fast production by the manufacturing industry. This leads to the short life span of electronics making them becomes outdated at a swiftly escalating rate around the world [10,11] and as a result of this, they are being dumped into the environment by the users. All of these lead to the release of heavy metals into water bodies and the environment at large which needs to be removed. Heavy metal removal by classical techniques involves expensive methodologies due to high energy and reagent requirements [12]. Numerous fresh approaches to cleaning the surroundings are being developed and many are already in use as an option to physical methods which are relatively too expensive [13].

Improvement in scientific methods and industrial technology has led to the utilization of intrinsic traits of natural resources which include microorganisms, to swiftly bio-absorb harmful inorganic contaminants to safe levels in water and soils. The safe levels are as determined by environmental health regulatory bodies to overcome environmental damages due to pollution [14]. This research aims to determine the bio-sorption efficiency of five fungal species on toxic metals present in electronic waste (e-waste) polluted water using agro-waste formulated media.

2. MATERIAL AND METHODS

2.2 Description of the Study Site

The site of the study was Alaba International Market, Ojo, Lagos State with a Latitude of 6°23'N and a Longitude of 2°42'E (Figure 1). This is the largest market in Africa and it's known for the sales of new and used electronic devices. Around the market are residential quarters and illegal dumpsites which consist of abandoned and outdated electronics which are dismantled and discarded for crude recycling. Thus, leaving the rest of the fragments to

Comment [GA9]: Could arise from. Not a definite statement.

Comment [GA10]: Reference for the source of this division.

Comment [GA11]: Full names and then symbols

Comment [GA12]: Too long sentence. Re-read and punctuate. And insert a reference please.

Comment [GA13]: Human body or animal body?

Comment [GA14]: Propotion or concentration or both?

Comment [GA15]: It will be best to capture further examples of 'dangerous health consequences. Especially from metals in questions – short term/acute effects and long term/chronic effects and then bioaccumulation potential etc.

Comment [GA16]: Another very long sentence. The opening sentence says there is a short life span of electronic products – definitive statement. Then the second sentence says 'This leads to the short life span' Rerword please.

Comment [GA17]: 'have a potential to release heavy metals. Also best to describe the connection between electronics and heavy metals.

Comment [GA18]: Can you give examples of classifiable techniques?

Comment [GA19]: Can you give any examples?

Comment [GA20]: Reference?

Comment [GA21]: How do you seek to achieve this aim? To put it differently, what are the objectives of the study?

Here, the physical and chemical analyses is not introduced and dthen suddenly appears later.

Comment [GA22]: Who is responsible for this? Reference.

be burnt (Plate 1) to reduce the waste volume, which tends to contaminate streams and well waters in the neighbourhood located near the e-waste open dumpsites. Wells and Streams which served the purpose of cooking, drinking and ablution as well as other domestic and commercial purposes by the inhabitants and workers in the area are situated very close to the e-waste open dumpsites.

Comment [GA23]: You should elaborate on the ways contamination gets to wells? Runoffs? Percolation to ground water? Deliberate introduction? Etc.

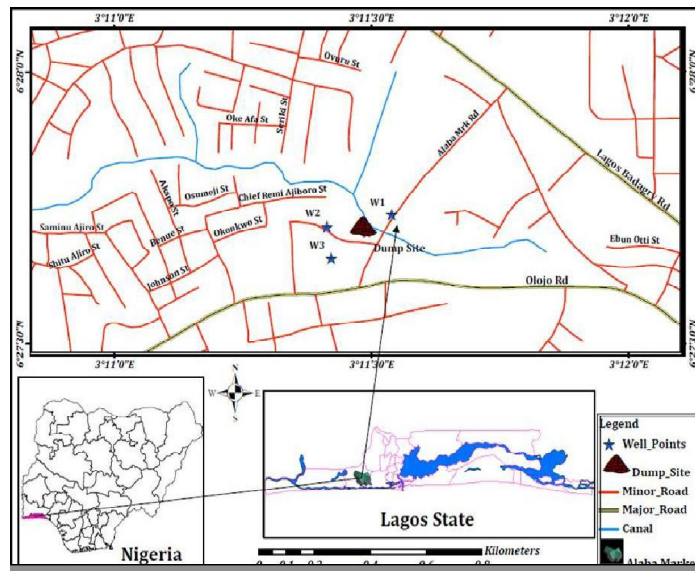


Fig. 1 Geographical View of Alaba International Electronics Market e-waste Dumpsite



Plate 1: Typical Activities at the e-waste Recycling Sites Where Samples were Collected

Comment [GA24]: Did you take this photo in the project location? If yes, state. If no, give credit.

2.2 Collection of Electronic Wastewater

Samples were collected from electronic waste polluted water site (EWPWS) near the dumpsite of e-waste in Alaba International market. The samples were stored in sterile bottles pre-washed with HNO₃ and rinsed thoroughly with deionized water. All samples were maintained at 4 °C in an ice box during transportation from the collection site to the laboratory [15]

Comment [GA25]: State comprehensive sampling methods.

2.3 Isolation and Morphological Identification of Fungi

Serial dilution was performed on the water samples by introducing 1ml sample into 9ml of distilled water until diluents of 10⁻¹ to 10⁻⁵ was achieved in separate test tubes. 1ml of different concentrations of the diluents were introduced into separate plates in the pour plate method, then potato dextrose agar was added to the plates, and the plate was swirled gently and allowed to solidify. Plates were incubated at 30±2°C for 72 hours. Different colonies of each of the organisms were sub-cultured into newly prepared Potato dextrose agar plates and incubated as mentioned earlier. Pure culture of each of the organisms was obtained and then stored at 4°C [16]. Morphological Identification of the Isolated fungi was performed using a bright field binocular Microscope. The cellular morphology and microscopic features of each of the isolates were observed and recorded [17,18]

Comment [GA26]: Specify what specific morphological features were examined and how each was used to identify the fungal strains. I take it that the fungal cells were indigenous in the waste water right? How sure can we be of the baseline quantities of organisms before contamination, their survival in physiological properties within contamination?

2.5 Medium Formulation Trials (MFT)

Brewery waste and honeycomb extracts were used for the media formulation trials (MFT) for the biosorption processes. A series of trial tests were performed to know the optimum volume of brewery waste extract, honeycomb extract and polluted water required for the process.

Comment [GA27]: Be more elaborate please. What 'series' of trial tests? Whose method is this and what is the value of the trial test on the research method? Elaborate.

2.6 Heavy Metals Biosorption from e-waste Polluted Water

For the biosorption process, the best media trial concentrations were used according to a modified method [19, 20]. Biosorption of heavy metals was done in ten flasks containing 200 ml of the e-waste polluted water, and 100 ml each of brewery waste and honeycomb extracts. The flasks were first sterilized using an autoclave and allowed to cool down. Thereafter, five fungal discs for each of the isolates were introduced into the medium, while the control was without fungi discs.

Comment [GA28]: Again elaborate. Before giving credit. Simply pointing to the reference means the reader has to abandon your work, go source for the reference before continuing. Readers are not the researchers. They are final users of your materials.

The experiment was carried out for 28 days and incubated in a shaker incubator at 25 °C. Samples were taken for analysis using Atomic absorption spectrophotometry (AAS) for each of the heavy metals (Pb, Cd, Cr, Ni, Zn, Cu, Fe) at seven days intervals throughout the incubation period using a modified method [19]. The percentage heavy metal (HM) removal was calculated using the equation below. Experiments were performed in triplicate while the average mean value was calculated as (X±SEM).

Comment [GA29]: At what temperature and for how long? How long was the cooling down for? Were the flasks kept in a sterile place during cooling down? Aain, elaborate.

$$\% \text{ HM Removal} = \frac{\text{HM (untreated water sample)} - \text{HM (treated water sample)}}{\text{HM (untreated water sample)}} \dots\dots\dots 1.1$$

Comment [GA30]: I am not clear with this methodology. Ideally, you measure the concentration of metals before treatment. Introduce pure strains to the contaminated water and measure concentrations post inoculation. By this method, how do you correct for volume differences in the untreated water and treated water volumes? Your methods have not stated that standard or consistent volumes were used. Similarly what controls; positive or negative have you introduced to the set up?

2.7 Physicochemical Analysis of e-waste Sample

The following physicochemical parameters such as pH, Electrical conductivity (EC), Total solid (TS), Dissolved oxygen (DO), Total suspended solids (TSS), Biological oxygen demand (BOD), Carbon oxygen demand (COD), Sulphate, Nitrate, Iron (Fe), Lead (Pb), Cadmium (Cd), Chromium (Cr), Copper (Cu), Zinc (Zn), Nickel (Ni), were determined for each of the experimental set-ups [21].

Comment [GA31]: What is the usefulness of the physical and chemical analyses? Does it require clean water for organisms to thrive and accumulate the metals? Mention the importance of this aspect of the study.

2.8 Heavy Metals Analysis of the e-waste Polluted Water

To determine the heavy metal concentration 100 ml of the samples were introduced into Pyrex beakers containing 10 ml of concentrated HNO₃. It was gently heated on a hot plate to reduce the volume and to discharge metals that are organic bounded, particulate or adsorbed on particulates. The beakers were allowed to cool. Thereafter another 5 ml of Conc. HNO₃ was added. It was heated again with the addition of Conc. HNO₃, as required till digestion, was completed.

Comment [GA32]: Whose method is this? Give credit. Additionally, this should be the first segment of the methodology. And now that we are here, clarify, if the isolated organisms were already acclimatized within the contaminated water, how can you say confidently that they reduced the metals? How can another researcher or interested persons reproduce this research and method?

The samples were finally evaporated to dryness; followed by the addition of 5 ml of HCl solution (1:1 v/v) and heated again. The addition of 5 ml of 5 M NaOH was done, then filtered. The filtrates were decanted into 100 ml volumetric flasks and diluted to the mark with distilled water. The digested samples were analyzed using a Buck Scientific Flame Atomic Absorption Spectrophotometer Thermo M5 Model [22, 23] in duplicate for the determination of heavy metals and the mean result was recorded. Fourier transform infrared (FTIR) was used to monitor the biological interactions of the fungi with the heavy metal polluted water sample.

Comment [GA33]: Whose method is this?

2.9 Statistical Analysis

Data collected were arranged using Excel spreadsheet 2010 and results were presented as percentages. Statistical Package for Social Sciences (SPSS) software for Windows (V. 21.0) was used for the data analysis.

Comment [GA34]: Whose method? Give credit.

3. RESULTS AND DISCUSSION

The result of the physicochemical parameters of the effluent shows that it has a pH of 4.5. The metals present include Lead (13.30 mg/L), Cadmium (16.50 mg/L), Chromium (6.41 mg/L), Nickel (3.81mg/L), Zinc (8.85 mg/L), Copper (8.33 mg/L), Manganese (3.65 mg/L), Sodium (150 mg/L), Potassium (18.55 mg/L), Calcium (210 mg/L), Magnesium (30 mg/L), Iron (5.60 mg/L). Moreover, the result of showed that it contains TS (550 mg/L), TSS (25.9mg/L), DO (2.5 mg/L), BOD (18.33 mg/L), COD (33.15 mg/L), Phosphate (11.33 mg/L), Sulphate (580 mg/L), Nitrate (65.30 mg/L) and EC (134.35 µs/cm) as seen in Table 1

Table 1. Physico-chemical analysis of e-waste polluted water

Comment [GA35]: Standing alone.

Parameters	Value (mg/L)
pH	6.8
Pb	13.3
Cd	16.5
Cr	6.41
Ni	3.81
Zn	8.85
Cu	8.33
Mn	3.65
Na	4.65
K	18.55
Ca	6.85
Mg	7.2
Fe	5.6
Total solid	55
Total suspended solids	25.9
Turbidity	very turbid
Dissolved Oxygen	6.8
Biochemical Oxygen demand	18.33
Chemical Oxygen demand	33.15
Phosphate	11.35
Sulphate	13.7
Nitrate	2.32
Electrical conductivity ($\mu\text{s}/\text{cm}$)	134.35

3.1 Isolation, Identification and Characterization of Isolates

A total of five (5) fungal species were isolated and identified to be: *Trichoderma harzianum*, *T. viride*, *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum*. The cellular morphological description is presented in Table 2 below.

Table 2. Morphological Description of the Isolated Fungi used for the Biosorption Studies

Fungal Identity	Description
<i>Trichoderma</i> spp. AG1	Colony at 20°C attained a diameter of 4.5-7.5 cm in 5 days. Initially more or less hyaline, later becoming whitish green in tufted conidia areas in blue-green shades. Reverse colourless. Conidiophores pyramidally branched.

<i>Trichoderma</i> spp. AG2	Colony at 20°C attaining a diameter of more than 9 cm in 5 days, more or less hyaline, whitish-green becoming olive-green in tufted conidia areas. Reverse colourless. Conidiophores pyramidally branched. Chlamydo spores were present in mycelia of older colonies, intercalary, sometimes terminal, mostly globose, hyaline and smooth-walled.
<i>Aspergillus</i> spp. AG1	Colonies at 25°C attained a diameter of 4-5 cm within 7 days. Consist of a compact white or yellow basal felt having a dense layer of dark brown to black conidiophores. Vesicles globose to sub-globose.
<i>Aspergillus</i> spp. AG2	Colonies at 25°C attained a diameter of 3-5 cm within 7 days. Consist of a dense felt of yellow-green conidiophores. Colonies on MEA are thinner but sporulating densely.
<i>Fusarium</i> spp.	It is white often with a peach tinge. Aerial mycelium floccose, whitish or peach which often changes to brownish (14-21 days). Sporodochia is absent. Conidia 3-5 cm within 7 days. Septate fusiform straight or somewhat curved, wedged shaped, apical cell beaked.

Comment [GA36]: Delete

3.2 Biosorption Studies of Heavy Metals Present in the e-waste Samples

3.2.1 Percentage Removal of Lead

The lowest biosorption value of lead by *A. niger* was recorded on day 7 (18.33 %), while the highest biosorption value was recorded on day 28 (70.34 %). For lead, the lowest biosorption by *A. flavus* was observed on day 7 (16.37 %), while the highest biosorption was recorded on day 28 (65.79 %). Also, the lowest biosorption of lead by *T. viride* was on day 7 (24.78 %), while the highest biosorption was found on day 28 (71.16 %). *T. harzianum* gave the least biosorption of lead on day 7 (23.21 %), while the highest biosorption was recorded on day 28 (84.35 %). *F. oxysporum* recorded the least biosorption of lead on day 7 (20.69 %), while the highest biosorption was recorded on day 28 (71.25 %).

Moreover, *A. flavus* had the lowest biosorption on day 7 (16.37 %) when compared with other organisms while *T. viride* gave the highest biosorption on day 7 (24.78 %). On day 14, *A. flavus* had the lowest biosorption (21.56 %), while *T. viride* gave the highest biosorption (40.47 %). *A. flavus* recorded the least biosorption on day 21 (34.23 %), while the highest

biosorption was recorded on day 21 by *T. harzianum* (56.12 %). *A. flavus* recorded the least biosorption on day 28 (65.79 %), while the highest biosorption was recorded on day 28 by *T. harzianum* (84.35 %) as found in Table 3.

Generally, lead biosorption increased as the time extended, where the highest biosorption percentages were observed at the 28th day of the experiment. *T. viridae* showed the highest biosorption capacities on the 7th and 14th days of treatment, which were 24.78 and 40.47%, respectively. On the other hand, *T. harzianum* recorded the highest biosorption of this metal on the 21st and 28th days, where the values were 56.12 and 84.35%, respectively.

Comment [GA37]: Put below the table Three.

Table 3. Percentage removal of lead (Pb) by different fungal isolates

Organisms/Day	% Biosorption at different days			
	D-7	D-14	D-21	D-28
<i>A. niger</i>	18.33	28.32	43.61	70.34
<i>A. flavus</i>	16.37	21.56	34.23	65.79
<i>T. viride</i>	24.78	40.47	50.67	71.16
<i>T. harzianum</i>	23.21	34.35	56.12	84.35
<i>F. Oxysporum</i>	20.69	26.47	42.36	71.25

Comment [GA38]: Might be better presented in a chart/line graph to show reduction over days.

3.2.2 Percentage Removal of Cadmium

In Table 4, *A. niger* gave the minimum biosorption value on day 7 (17.02 %), and the maximum value was given on day 28 (66.74 %). *A. flavus* gave the minimum value on day 7 (20.33 %), and the maximum value was given on day 28 (63.88 %). *T. viride* gave the minimum value on day 7 (26.45 %), and the maximum value was given on day 28 (73.18 %). Also, *T. harzianum* gave the minimum value on day 7 (31.10 %), and the maximum value was given on day 28 (80.76 %). *F. oxysporum* gave the minimum value on day 7 (16.95 %), and the maximum value was given on day 28 (65.66 %). However, on day 7, *A. niger* (17.02 %) had the least value while the highest was given by *T. harzianum* (31.10 %). On day 14, *A. flavus* (22.21 %) had the least value while the highest was given by *T. harzianum* (40.78 %). On day 21, *A. niger* (28.88 %) had the least value while the highest was given by *T. harzianum* (59.80 %). On day 28, *A. flavus* (63.88 %) had the least value while the highest was given by *T. harzianum* at 63.88 and 80.76 % respectively.

Comment [GA39]: Same comment as above

Table 4. Percentage removal of Cadmium (Cd) by different fungal isolates

Organisms/Day	% Biosorption at different days			
	D-7	D-14	D-21	D-28
<i>A. niger</i>	17.02	22.55	28.88	66.74
<i>A. flavus</i>	20.33	22.21	34.54	63.88
<i>T. viride</i>	26.45	36.46	46.91	73.18
<i>T. harzianum</i>	31.10	40.78	59.80	80.76
<i>F. Oxysporum</i>	16.95	23.65	29.64	65.66

Comment [GA40]: Same comment as above

3.2.3 Percentage Removal of Chromium

Comment [GA41]: Same comment as above

Table 5 showed that *A. niger* recorded the minimum biosorption (22.92 %) on day 7 (D-7) while the maximum value was recorded on day 28. *A. flavus* recorded the minimum value on day 7 (21.22 %) while the maximum value was recorded on day 28 (61.48 %). *T. viride* recorded the minimum value on day 7 (32.91 %) while the maximum value was recorded on day 28 (94.32 %). *T. harzianum* recorded the minimum value on day 7 (45.68 %) while the maximum value was recorded on day 28 (96.30 %). *F. oxysporum* recorded the minimum value on day 7 (31.12 %) while the maximum value was recorded on day 28 (78.77 %). Furthermore, on day 7, *A. flavus* (21.22 %) had the least value while the highest was given by *T. harzianum* (45.68 %). On day 14, *A. niger* (23.74 %) had the least value while the highest was given by *T. harzianum* (49.41 %). On day 21, *A. niger* (36.00 %) had the least value while the highest was given by *T. harzianum* (72.00 %). On day 28, *A. flavus* had the least chromium removal while the highest was recorded by *T. harzianum* at 61.48 and 96.30 % respectively.

Table 5. Percentage removal of Chromium (Cr) by different fungal isolates

Comment [GA42]: Same comment as above

Organisms/Day	% Biosorption at different days			
	D-7	D-14	D-21	D-28
<i>A. niger</i>	22.92	23.74	36.00	61.73
<i>A. flavus</i>	21.22	34.58	38.67	61.48
<i>T. viride</i>	32.91	46.25	66.44	94.32
<i>T. harzianum</i>	45.68	49.41	72.00	96.30
<i>F. Oxysporum</i>	31.12	39.53	48.89	78.77

3.2.4 Percentage Removal of Nickel

Comment [GA43]: Same comment as above

Table 6 showed that Day 7 recorded the lowest biosorption value (45.15 %) by *A. niger* while the highest value was noticed on day 28 (83.27 %). The lowest value by *A. flavus* was recorded on day 7 (56.29 %) while the highest value was on day 28 (92.49 %). Day 7 (67.14 %) recorded the lowest value by *T. viride* while the highest value was found noticed on 28 (95.22 %). Day 7 (72.29 %) recorded the lowest value by *T. harzianum* while the highest value was on day 28 (94.88 %). Day 7 (58.57 %) recorded the lowest value by *F. oxysporum* while the highest value was recorded on day 28 (89.42 %). Also, on day 7, *A. niger* (45.14 %) had the least value while the highest was given by *T. harzianum* (72.29 %). On day 14, *A. niger* (45.51 %) had the least value while the highest was given by *T. harzianum* (78.02 %). On day 21, *A. niger* (58.09 %) had the least value while the highest was given by *T. viride* (86.47 %). On day 28, *A. niger* had the least nickel removal (83.27 %) while the highest was recorded by *T. viride* (95.22 %).

Table 6. Percentage removal of Nickel (Ni) by different fungal isolates

Comment [GA44]: Same comment as above

Organisms/Day	% Biosorption at different days			
	D-7	D-14	D-21	D-28
<i>A. niger</i>	45.14	45.51	58.09	83.27
<i>A. flavus</i>	56.29	63.78	76.24	92.49
<i>T. viride</i>	67.14	74.92	86.47	95.22
<i>T. harzianum</i>	72.29	78.02	82.51	94.88
<i>F. Oxysporum</i>	58.57	75.54	80.86	89.42

3.2.5 Percentage Removal of Zinc

Comment [GA45]: Same comment as above

A. niger recorded the minimum biosorption value on day 7 (26.16 %) while the maximum value was recorded on day 28 (81.97 %). *A. flavus* recorded the minimum value on day 7 (18.81 %) while the maximum value was recorded on day 28 (68.26 %). *T. viride* recorded the minimum value on day 7 (38.02 %) while the maximum value was recorded on day 28 (86.29 %). *T. harzianum* recorded the minimum value on day 7 (54.77 %) while the maximum value was recorded on day 28 (92.10 %). *F. oxysporum* recorded the minimum value on day 7 (30.67 %) while the maximum value was recorded on day 28 (69.75 %). In addition, on day 7, *A. flavus* (18.81 %) had the least value while the highest was given by *T. harzianum* (54.77 %). On day 14, *A. flavus* (27.55 %) had the least value while the highest was given by *T. harzianum* (63.64 %). On day 21, *F. oxysporum* (49.42 %) had the least value while the highest was given by *T. harzianum* (73.55 %). On day 28, *A. flavus* (68.26 %) had the least value while the highest was given by *T. harzianum* (92.10 %). It was observed that throughout the removal process, *Trichoderma spp.* gave the best result most especially *T. harzianum* compared with other organisms such as *A. niger*, *A. flavus* and *Fusarium oxysporum* used in the biosorption process. This can be seen in Table 7.

Table 7. Percentage removal of Zinc (Zn) by different fungal isolates

Comment [GA46]: Same comment as above

Organisms/Day	% Biosorption at different days			
	D-7	D-14	D-21	D-28
<i>A. niger</i>	26.16	50.71	67.34	81.97
<i>A. flavus</i>	18.81	27.55	53.47	68.26
<i>T. viride</i>	38.02	57.85	67.49	86.29
<i>T. harzianum</i>	54.77	63.64	73.55	92.10
<i>F. oxysporum</i>	30.67	42.15	49.42	69.75

3.3 Biosorption Study Using Fourier-transform Infrared (FTIR) Spectroscopic Techniques

FTIR analysis was carried out to study the interaction between the fungal isolates and the contaminants in the e-waste-polluted water samples. The functional groups were used in monitoring this. Exactly 5 ml of each of the samples drawn on days 7, 14, 21, and 28 of the experimental set-ups were smeared on the attenuated total reflectance (ATR) and mounted on the machine. The smears obtained were analyzed with a Shimadzu IR Affinity-1S FTIR spectrophotometer covering a frequency of 4000-500 cm^{-1} . The machine was directed to analyze after all the parameters have been set. The result of Fourier Transform Infrared (FTIR) carried out showed that the fungal isolates used were able to cause vibration in absorption frequencies thereby leading to the disappearance and or reduction of their absorption frequencies. The peaks on the control experiment where no fungal discs were used are: 455.56 cm^{-1} , 1035.77 cm^{-1} , 1462.04 cm^{-1} , 1637.56 cm^{-1} , 2019.47 cm^{-1} , 2200.78 cm^{-1} , 2360.87 cm^{-1} , and 3327.21 cm^{-1} while that of day 28 were 432.05 cm^{-1} , 513.07 cm^{-1} , 827.46 cm^{-1} , 1001.06 cm^{-1} , 1635.64 cm^{-1} , 1975.11 cm^{-1} , 2166.06 cm^{-1} , and 3317.56 cm^{-1} as can be seen in Fig. 2 and Fig. 3 respectively.

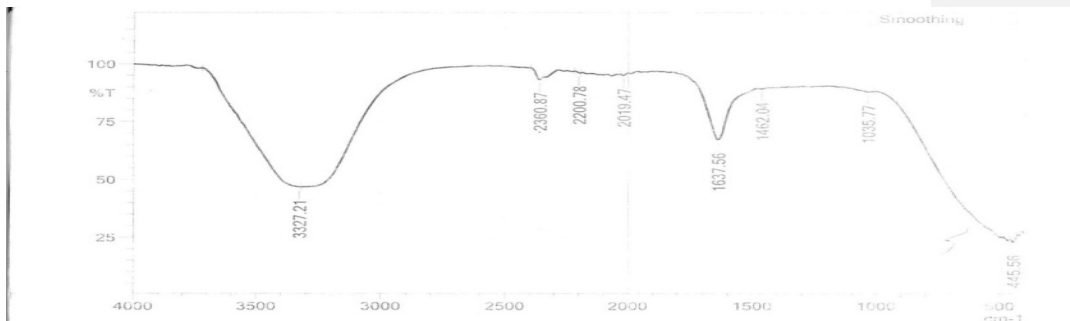


Fig. 2 FTIR of Control Experiment without fungal discs

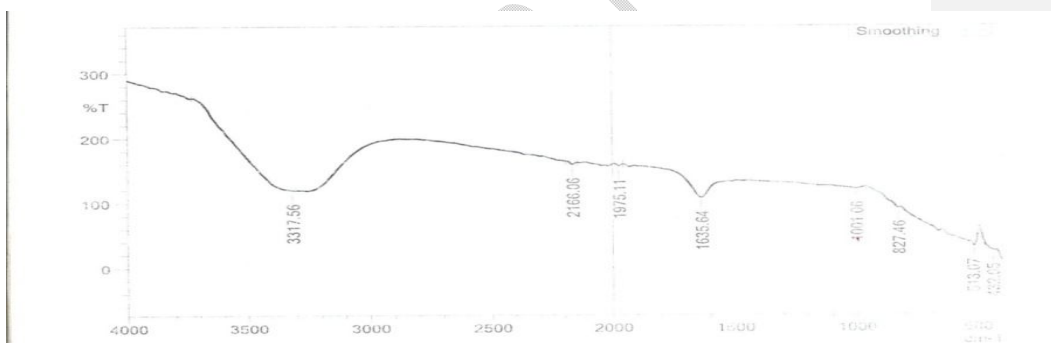


Fig. 3 FTIR at Day 28 of biosorption study with fungal discs

3.4 Biosorption Efficiency

It was evident from this work that extracts from brewer-spent grain and honeycomb used as carbon sources were observed to greatly support *Aspergillus niger*, *A. flavus*, *Trichoderma viride*, *T. harzianum* and *Fusarium oxysporum* in the removal of Pb, Cd, Cr, Zn and Ni. Industrial activities such as electrical waste, technological development and so on have led to the continuous discharge of heavy metals into the environment posing a significant threat to public health worldwide. Fungi have been reported to be a far better choice for bioremediation of metal [24]. Fungi can accommodate high concentrations of heavy metal build-up without having toxic effects [25]. Hence, fungi selected for this research work have shown a unique ability to accumulate metal; they were also very easy to culture in microbiological media [26]. *Trichoderma harzianum* and *T. viride* showed higher biosorption

Comment [GA47]: Have you started discussion here?

of lead on day 28 when compared to other microorganisms which were *Aspergillus flavus*, *A. niger* and *Fusarium oxysporum*. *Aspergillus niger*, *A. flavus* and *F. oxysporum* showed low biosorption of lead compared to other isolates. *T. harzianum* with high lead uptake indicates that it has more binding sites on its cell wall thus acting as a biosorbent in the removal of lead from industrial effluent containing higher lead concentration [27]. Similar results have been reported for the uptake of lead by different fungi [28,29,30]

Comment [GA48]: This section reads like what should be in the conclusion.

Zinc has been applied in a broad diversity of cellular processes and is obligatory for retaining macromolecule structural stability, therefore, serving as a co-factor for about 300 enzymes [31]. In this study, *A. flavus* and *F. oxysporum* recorded lower biosorption levels of zinc on day twenty-eight. This reduction could be due to the toxic concentration of heavy metals by the electrical waste or an increase in a high level of tolerance and biosorption properties of *F. oxysporum* and *A. flavus*. This result agrees with the findings [32] that reported that some heavy metals' toxicity in fungi is a result of their strong binding affinities with the cell membrane which causes loss of structural integrity and impairment of cell function. *Aspergillus niger*, *Trichoderma viride*, and *Trichoderma harzianum* showed an important increase in the biosorption of zinc but lower compared to *Aspergillus flavus* and *Fusarium oxysporum*. Similar results have been postulated in the use of *Aspergillus niger*, and *Penicillium chrysogenum* as biosorbent in the removal of Zinc

Comment [GA49]: Is zinc present in electronics? Which electronics.

Nickel is recognized as a chief ecological contaminant. It has clastogenic, toxic and carcinogenic effects [33]. In this study, *Aspergillus niger*, and *Fusarium oxysporum* have lower biosorption levels for lead (Pb) compared to *Aspergillus flavus*, *Trichoderma viride* and *Trichoderma harzianum*. The decline observed in *Aspergillus niger* and *Fusarium oxysporum* can be due to a decrease in biosorption of the microbes from the ecological surroundings which may be due to microbe-fungi interactions of metals in fungal cell wall as postulated [34]. The report also showed that the adsorption of heavy metals is controlled by a fungi cell wall and is used for future clean-up of pollutants from the environment. Similar findings were made by researchers [19] who reported biosorption of Nickel by *Bacillus subtilis*, *Micrococcus luteus* and *Trichoderma harzianum*. In addition, other researchers [32] have also reported the use of *Aspergillus niger* and *A. flavus* in the biosorption of nickel. Similar results in tandem with the acceptance of Nickel by fungi have also been acknowledged in literature [35, 36]

Comment [GA50]: Is nickel part of electronics? What is it used for in the electronics industry and what is the prevalence of this metal normally? This question should have been cleared if the background covered the topic of the uses of the different metals in the electronics industry and to what extent they are used.

Cadmium has been postulated by many researchers to have a renowned biological function if bio-available. Cadmium causes toxicity to cells by interacting with nucleic acids thereby producing reactive oxygen species that cause oxidative damage which leads to respiratory problems [37]. In this research, it was observed that the biosorption of cadmium by *A. niger*, *A. flavus* and *Fusarium oxysporum* followed the same trend all through the period of the experiment. This finding agrees with the other reports [32] that *A. flavus* and *A. niger* showed almost the same cadmium uptake ability during the biosorption of heavy metals. *Trichoderma viride* and *T. harzianum* recorded the highest level of biosorption of cadmium on day twenty-eight which could be due to a higher amount of cadmium uptake by the biomass compared to *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum* due to the electrostatic interactions of the cell surface functional groups. These findings correlate with the report [26] that *T. viride* and *T. harzianum* showed higher amounts of cadmium uptake compared to other related microorganisms. Similar findings were also reported [38] that *Trichoderma* species have bioremediation effectiveness and their biosorption ability differs depending on the species. They are also known to be important fungi in decreasing Cadmium ions. Moreover, similar results which have been published about the uptake of cadmium by fungi have also been reported [39, 40, 41, 42]

Comment [GA51]: Same comment as above.

Chromium is a crucial element helping the body to make use of essential elements. Extreme ingestion or exposure can lead to damage to the body. It has also been reported that *A. niger*, *A. flavus* and *Trichoderma viride* have been used in removing chromium from metal-contaminated wastewater [32, 43]. The use of *Bacillus cereus* in the removal of chromium from effluent has also been reported [44]. Similar findings to the uptake of chromium by fungi have also been documented [45, 46, 47, 48]. In this study, *Trichoderma viride* and *T. harzianum* showed higher biosorption compared to *Aspergillus niger*, *A. flavus* and *Fusarium oxysporum*.

Comment [GA52]: Same comment as above.

4.0 CONCLUSION

In summary, the ability of the fungal isolates to bind and reduce heavy metals from e-waste polluted water, especially *Trichoderma harzianum* was established. It recorded a very high tolerance for biosorption of heavy metals which is an indication that it is a resourceful biological agent that can be harnessed for toxic environmental contaminants removal.

In summary, it is essential that heavy metals be removed from polluted water body to avoid its deleterious effects on public health. This can be achieved biologically using any of the fungi isolated in this work especially *Trichoderma harzianum*.

Comment [GA53]: Again, the physico chemical analyses found no bearing in the entire study. No usefulness.

The starting point ought to have been a background on the prevalence of specific metals from the electronics in Alaba and environs citing references or field studies that identified the metals. There ought to have been a truly documented public health concern that requires this intervention.

Finally, look through the objectives and ensure the conclusion addresses all the objectives.

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Comment [GA54]: Limit your references to only those cited in the text.

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